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Biological Resources

ENDOCRINE DISRUPTION WEBINAR

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*Cooperating agencies:

- U.S. Geological Survey
- Texas Tech University
- Texas Parks and Wildlife Department
- The Wildlife Management Institute
- Fish and Wildlife Service

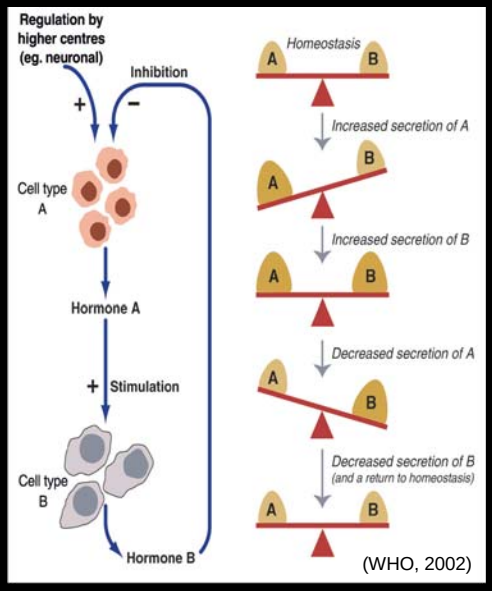
Objectives of Webinar

- To define Endocrine Disruption (ED)
- To review field and laboratory approaches to assess ED in fish and wildlife
- To discuss management implications of results obtained from ED research

Endocrine Systems

- An Endocrine System is made up of endocrine glands or cells that secrete their products (hormones) into their surrounding space; for example, extracellular space or circulatory system. Endocrine glands are therefore “ductless glands.”
- Basic difference between “Endocrine” and “Exocrine” glands is that the latter deliver their products (non-hormones, typically) to their destination via a duct. For example, sweat glands, salivary glands, et cetera.

Endocrine Systems

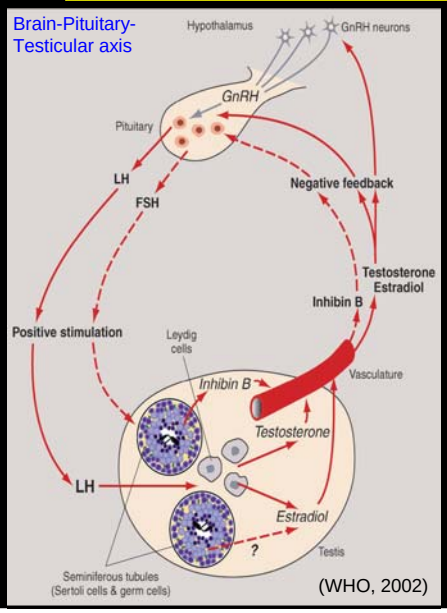


(WHO, 2002)

Diagram showing typical regulation of hormone production by an endocrine gland.

- Example:
  - ✓Higher center = Brain
  - ✓Cell type A = pituitary gland (“master gland”)
  - ✓Cell type B = thyroid gland
- Negative feedback regulation by Hormone B (e.g., thyroid hormone) maintains “homeostasis”

Endocrine Systems



(WHO, 2002)

Diagram showing endocrine regulation of testicular function in mammals.

- Brain produces GnRH (Hormone A)
- GnRH stimulates production of gonadotropins by pituitary gland (LH and FSH; Hormone B)
- Gonadotropins regulate testicular development and production of steroid hormones and other endocrine factors such as Inhibin (Hormone C)
- Negative feedback regulation by Hormone C (steroids, inhibin) maintains constant levels of all hormones ("homeostasis") so that testicular function can proceed normally.

So what is an Endocrine Disruptor?

- Toxicant (poison)
  - ✓ Chemical or mixture of chemicals that presents a risk of death, disease, injury, or birth defects in organisms that ingest or absorb it (National Institutes of Health)
  - ✓ Natural toxicants are also called toxins
  - ✓ Other toxicants can be classified in a number of ways:*
    - ➢ Source – animal, manufacturing, plant
    - ➢ Structure – metal, steroid, et cetera
    - ➢ Target organ – lever, gonads, et cetera
    - ➢ Effects (cancer, feminization, masculinization, et cetera)
- Endocrine-disrupting chemical (EDC)
  - ✓ Any class of toxicant that "disrupts" the endocrine system and its function

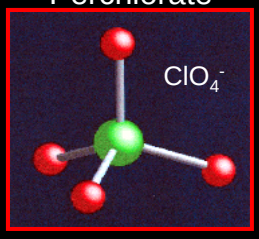
*Dickerson RL, Smith EE. 2006. Introduction to the science of toxicology. In, Endocrine Disruption (Norris DO, Carr JA, editors), Chapter 7, New York: Oxford University Press.

EDC Definitions

- "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and regulation of developmental processes" [USEPA (Kavlock et al.), 1997]
- "… chemical substance that interferes with, or has **adverse effects** on, the production, distribution, or function of …hormones" (Society of Environmental Toxicology and Chemistry, 2000).
- "… an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes **adverse health effects** in an intact organism, or its progeny, or (sub)populations" (World Health Organization, 2002).
- "… naturally occurring compounds or man-made chemicals that may interfere with the production or activity of hormones of the endocrine system leading to **adverse health effects**" (U.S. Public Health Service-NIEHS, 2006).
- Note that current definitions of EDC include a specific statement about **"adverse effects."** This is an important consideration when making management decisions based on EDC research (more later).

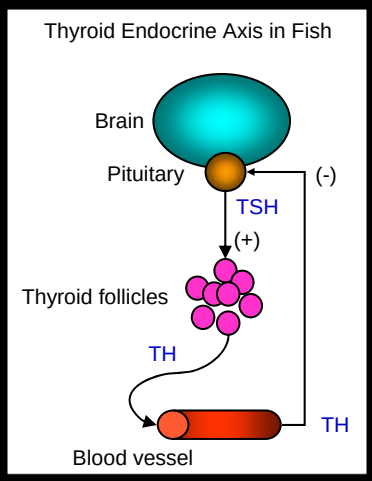
How Does an EDC Work? … … perchlorate as disruptor of TH synthesis

Perchlorate



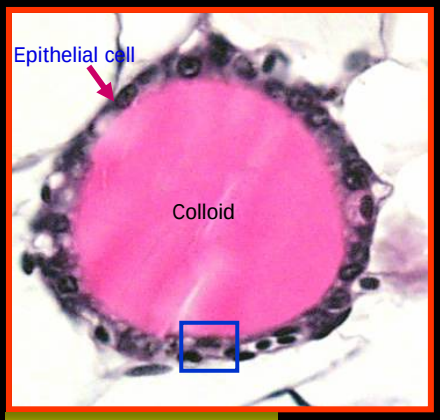
- Perchlorate – inhibitor of Thyroid Hormone (TH) synthesis

How Does an EDC Work? …
… perchlorate as disruptor of TH synthesis

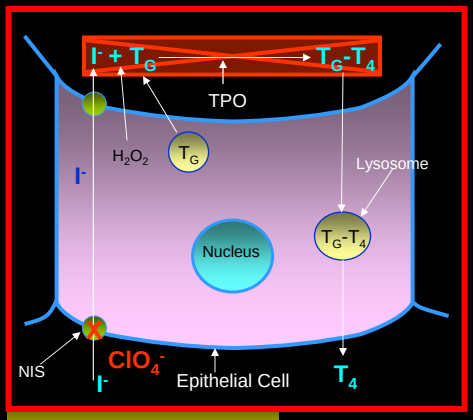


- TSH: Thyroid-Stimulating Hormone
- TH: Thyroid Hormone
- (+): stimulation
- (-): inhibition

How Does an EDC Work? …
… perchlorate as disruptor of TH synthesis

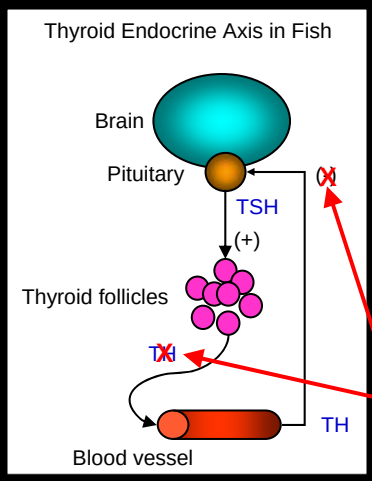


A. Thyroid Follicle



B. TH Synthesis

How Does an EDC Work? …
… perchlorate as disruptor of TH synthesis



- Perchlorate causes the loss of negative feedback and homeostasis
- Results of disruption include:
  - ✓Increased production of TSH
  - ✓Hyperstimulation of thyroid gland
  - ✓Development of Goiter
  - ✓Potential developmental and reproductive anomalies

Approaches to EDC Research

- Laboratory Studies
  - ✓ Main advantage: availability of “control” (untreated) conditions
  - ✓ Main disadvantage: can be far removed from “real-world” conditions
- Field Studies
  - ✓ Main advantage: reflect real-world conditions
  - ✓ Main disadvantage: lack of control conditions. “Reference” conditions or sites have to be used instead.
- Best approach – combination of laboratory and field studies
  (mesocosms studies are sometimes also used)

EDC Research in the Laboratory

- Standard (lethal) toxicity studies:
  - ✓ Used to determine relative lethal toxicity of chemicals:
    - ➢ LD50 (LC50): dose (or atmospheric or water concentration) of chemical required to kill 50% of the exposed subjects over a defined period of time
    - ➢ Parameters are obtained from dose- (concentration)-response curves
- Nonlethal (sublethal) toxicity studies:
  - ✓ Used to determine relative sublethal toxicity of chemicals:
    - ➢ ED50 (EC50): dose (or atmospheric or water concentration) of chemical required to have an effect ("effective dose") on a sublethal endpoint of interest
    - ➢ Parameters are also obtained form dose- (concentration)-response curves
    - ➢ Other useful parameters include:
      - ❖ Lowest-observed effect level/concentration (LOEL/LOEC)
      - ❖ No-observed effect level/concentration (NOEL/NOEC)
- Sublethal toxicity studies are the primary format for EDC research

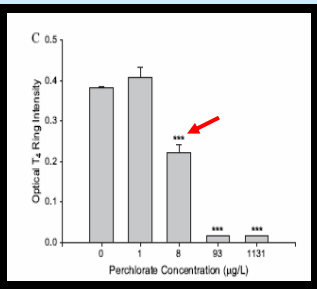
EDC Research in the Laboratory

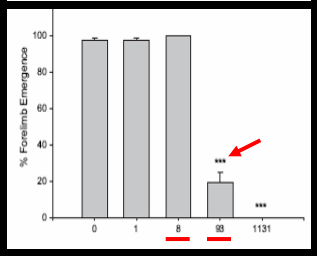
- Types of Laboratory EDC Research:
  - ✓ Exploratory (discovery) research
  - ✓ "Regulation/management research"

EDC Research in the Laboratory

- Example of exploratory research question:
  - ✓ What is the LOEC for thyroid effects of perchlorate in amphibians?

Xenopus laevis





Optical T4 ring is a measure of TH production in thyroid follicles of fish and amphibians [Mukhi et al. (2005), ETC 24:1107-1115; Hu et al. (2006), Toxicol Sci 93:268-277]

- LOEC of perchlorate on amphibian thyroid function = 8 ppb
- LOEC of perchlorate on tadpole development = 93 ppb

- There is no recommended safe level of perchlorate for freshwater aquatic habitats
- USEPA-recommended safe level for drinking water is 24.5 ppb

EDC Research in the Laboratory

- Laboratory methods conduct EDC research for the purpose of regulation have not yet been approved by the USEPA Endocrine Disruption Screening Program (EDSP)
- A 2-tiered approach is currently under consideration:
  - ✓ Tier 1: simple and complementary tests to efficiently screen for chemicals with the potential to affect androgen, estrogen and thyroid hormonal pathways.
    - ➢ Battery of in vitro and in vivo tests
  - ✓ Tier 2: more complex and definitive testing that would establish quantitative relationships between dose (concentration) and adverse effects.
    - ➢ 2-generation tests looking at developmental and reproductive endpoints (mammal, bird, fish, crustacean).

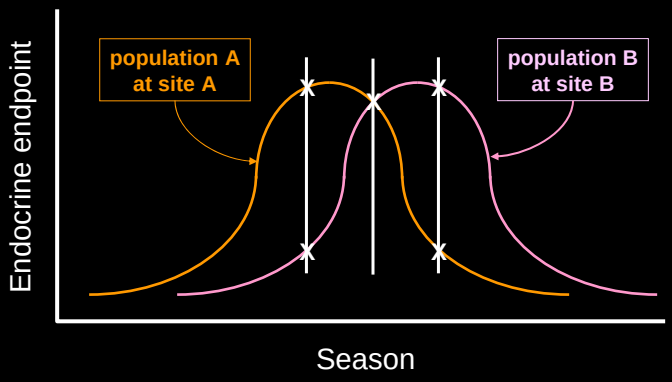
EDC Research in the Laboratory

- Tier 1 tests proposed by the USEPA in 2008:
  - ✓ In vitro tests
    - Estrogen receptor (ER) binding – rat uterus
    - Human ERα transcriptional activation – HeLa-9903 cell line
    - Androgen receptor (AR) binding – rat prostate
    - Steroidogenesis – H295R (human) adrenocortical carcinoma cell line
    - Aromatase (human recombinant)
  - ✓ In vivo tests
    - Uterotrophic (rat)
    - Hershberger (rat)
    - Pubertal female (rat)
    - Pubertal male (rat)
    - Amphibian metamorphosis (frog)
    - Fish short-term reproduction
- Formal proposal for Tier 2 tests has not yet been made.

EDC Research in the Field

- If species of interest is unavailable for sampling (e.g., because it is rare or listed), an appropriate sentinel species should be selected to match niche as close as possible
- Sampling design should include sites with suspected gradient of EDCs, including a reference site if one can be identified
- Suspected EDCs should be measured in environment (water, sediment, food chain) and in study species
- Because field studies are expensive, it is typically recommended that a pilot study be conducted before the more definitive, and more costly, definitive study
- Pilot and definitive field studies must consider that biological phenomena typically show a seasonal pattern that may be strongly influenced by environmental conditions other than the chemical environment

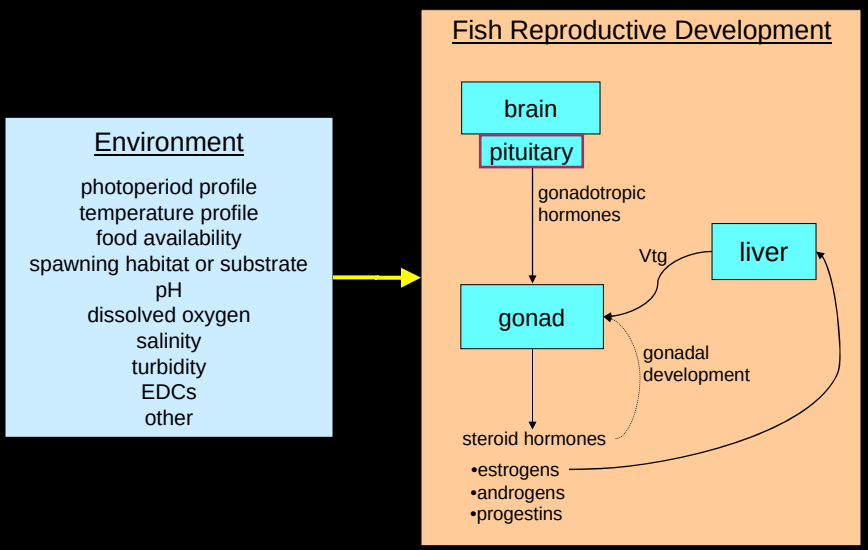
Seasonality of Biological Phenomena



- A > B
- A = B
- A < B

- This hypothetical scenario makes it clear that single-sampling designs could lead to different and even contradictory conclusions
- Seasonal shift in endocrine profile between populations or aquatic organisms may occur due to site differences in, for example, water temperature
- However, such drifts do not necessarily mean that there will be adverse effect

The Relationship Between the Environment and Reproduction is not Simple



The Relationship Between the Environment and Reproduction is not Simple

- Common Carp in Lake Mead*
  - ✓ Female fish
    - ➢ Differences in gonadal development between reference and polluted sites were attributed to differences in seasonal temperature profiles
    - ➢ There was some indication than female fish from the polluted site had unexpectedly higher fecundity, an observation tentatively attributed to the higher productivity of the site (which receives wastewater effluent)
  - ✓ Male fish
    - ➢ Differences in gonadal development and histopathologies between reference and polluted sites could only be attributed to the chemical (EDC) composition of the environment.
  - ✓ These observations clearly show that the non-chemical environment cannot be ignored in field studies of EDCs, and also only point to the possibility of gender-specific EDC effects

*Patiño et al. 2003. Morphometric and histopathological parameters of gonadal development in adult common carp from contaminated and reference sites in Lake Mead, Nevada. *J. Aquat. Animal Health* 15:55-68.

Regulatory and Management Considerations

- When dealing with humans,
  Target of interest typically is the individual – if my own endocrine or reproductive systems are being affected by some anthropogenic chemical I want this to stop, regardless of the risk to the human population as a whole
- When dealing with fish and wildlife,
  Target of interest typically is the population – biological resource management agencies are typically interested in the viability and stability of populations or subpopulations, not of individuals.
- This difference can make your job more difficult than, for example, the U.S. Public Health Service.

Regulatory and Management Considerations

Status of USEPA's EDC Screening Program

- **1996** – The Food Quality Protection Act of 1996 and subsequent amendments to the Safe Drinking Water Act and the Federal Food, Drug and Cosmetic Act required the USEPA to:
  - ✓ *develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in **humans** that is similar to an effect produced by a naturally occurring **estrogen**, or other such endocrine effect as the Administrator may designate [21 U.S.C. 346a(p)].*
- **1998** – An Endocrine Disruptor Screening Test Advisory Committee (EDSTAC) formed by the USEPA recommended expansion of target organisms to include **fish and wildlife** and endocrine systems to include the **androgen and thyroid systems**
- **2007** – USEPA published draft list of 73 chemicals (pesticides) to be considered for Tier 1 screening under the Endocrine Disruptor Screening Program (EDSP)
- **2007** – USEPA published draft policies and procedures for the EDSP
- **2008** – Review of proposed ESDP Tier 1 screening assays for identification of **potential** Endocrine Disruptors was held in March 2008 by USEPA's FIFRA Scientific Advisory Panel
- **Today** – Twelve years after passage of the 1996 Food Quality Protection Act, endocrine disruptor screening tests have yet to be approved or implemented … but maybe soon!

Regulatory and Management Considerations

So how are/can regulatory and management decisions made?

- Two basic approaches
  - ✓ Weight of Evidence
  - ✓ Precautionary Principle

Regulatory and Management Considerations

Weight of Evidence – favored by U.S. agencies

- *"The weight-of-evidence approach is the process by which measurement endpoints are related to an assessment endpoint to evaluate whether a significant risk of harm is posed to the environment. The approach is planned and initiated at the problem formulation stage and results are integrated at the risk characterization stage"*
  (Massachusetts Weigh of Evidence Working Group, 1995)

- **Assessment endpoints** are explicit expressions of the actual environmental value that is to be protected. For example, achieving the necessary numbers of Razorback Suckers (or the appropriate habitat conditions) in the Colorado River that will result in stable fish populations.

- **Measurement endpoints** are the lines of evidence used to evaluate the assessment endpoint. For example, results of Tier 1 and 2 tests. Multiple measurement endpoints are often associated with a single assessment endpoint.

Regulatory and Management Considerations

Precautionary Principle

"Better safe than sorry." "An ounce of prevention is worth a pound of cure."

- *"In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."*
  Principle 15, United Nations Rio Declaration on Environment and Development (July 1992)

- *"The precautionary principle has been central to many of the debates concerning the appropriate approach to the threat posed by endocrine active substances …. This newly emerging principle has been applied to issues as diverse as persistent organic pollutants and the European trade barrier on beef from hormone-treated cattle."*
  Goldstein BD. 2003. Precautionary principle and endocrine active substances. Pure Appl Chem 75:2515-2519.

- *"I view risk assessment and precaution as complementary, rather than opposite approaches. There are many environmental health issues where uncertainties abound, and the role of endocrine active substances … and their consequences are fraught with uncertainties."*
  Gochfeld M. 2003. Why epidemiology of endocrine disruptors warrants the precautionary principle. Pure Appl Chem 75:2521-2529.

Thank You For Your Attention

Any Questions?